Synthesis, structures and antimicrobial activities of nickel(II) and zinc(II) diaminomaleonitrile-based complexes

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Abstract

Three Ni(II) and Zn(II) complexes [Ni(L1)], [Ni(L2)], and [Zn(L3)(DMSO)] (L1 = 2,3-bis(2-hydroxybenzylideneimino)-2,3-butenedinitrile, L2 = 2,3-bis(2-hydroxy-3-methoxybenzylideneimino)-2,3-butenedinitrile, L3 = 2,3-bis(2-hydroxy-1-naphthylideneimino)-2,3-butenedinitrile) were obtained in DMSO by one-pot syntheses. The complexes were characterized by physicochemical and spectroscopic methods. Also, their solid-state structures were determined by single-crystal X-ray diffraction. The geometries of the Ni(II) and Zn(II) complexes were square planar and square pyramidal, respectively. The complexes were screened in vitro against a fungal species and eight species of bacteria, revealing their antimicrobial activity.

Introduction

The synthesis and characterization of Schiff bases and their transition metal complexes has attracted considerable attention in recent decades [1–3]. Schiff base ligands with a variety of donor atoms including nitrogen, oxygen and sulfur can be coordinated with transition metal atoms. Depending on the type and oxidation state of the central metal atom and the ligand structure, they can be bidentate, tridentate, tetradentate or polydentate, and depending on the nature of the possible counter ion, transition metal Schiff's base complexes show various coordination modes with varying degree of distortion [4, 5]. Among these, the tetradentate Schiff bases with N₂O₂ coordination sites belong to ligands investigated most often in coordination chemistry [6–8]. The transition metal complexes formed from tetradentate Schiff bases exhibit a wide range of biological properties [9–12]. 2,3-Diamino-cis-2-butenedinitrile (diaminomaleonitrile: DAMN) is an attractive precursors to nucleotides and is considered as an intermediate in the synthesis of a wide variety of heterocyclic compounds [13]. The condensation of

DAMN with different aromatic aldehydes can form 1:1 and/or 1:2 Schiff base ligands [14]. In recent studies, we have reported the synthesis and characterization of complexes incorporating tridentate NNO-Schiff base ligands based on diaminomaleonitrile [15, 16]. In this project, our efforts were directed to the synthesis and characterization of nickel(II) and zinc(II) complexes with tetradentate N₂O₂-Schiff base ligands based on diaminomaleonitrile and various aldehydes such as 2-hydroxybenzaldehyde, 3-methoxy-2-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde.

Experimental

Materials and methods

All reagents for synthesis and analysis were purchased from Merck and used without further purification. Elemental analyses were recorded on a Thermo Finnigan Flash Elemental Analyzer 1112EA. Melting points were measured on an Electrothermal-9100 apparatus. The IR measurements were taken on a FTIR Tensor 27 infrared spectrophotometer as KBr disks in the range of 400–4000 cm^{−1}. ¹H NMR spectra were measured on a Bruker AVANCE BRX 250 MHz spectrometer using d₆-DMSO as solvent for the complexes. The chemical shift values (δ) are given in ppm. The electronic absorption spectra in DMSO solution were recorded by a Cary 50 UV–Vis spectrophotometer. Bacteria were isolated from clinical samples or purchased from Merck Company.

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Preparation of [Ni(L1)] and [Ni(L2)]

A solution of 2-hydroxybenzaldehyde (2.0 mmol, 0.21 ml) for H₂L1 and 3-methoxy-2-hydroxybenzaldehyde (0.304 g, 2.0 mmol) for H₂L2 and 2,3-diamino-cis-2-butenedinitrile (0.108 g, 1.0 mmol) in DMSO (5 ml) was heated for 30 min. Afterward, triethylamine (0.200 g, 2.0 mmol) and NiCl₂·6H₂O (0.238 g, 1.0 mmol) in DMSO (5 ml) were added dropwise and the resulting reaction mixture was refluxed for 4 h while stirring constantly. The clear green solution was allowed to stand several days at room temperature, providing blue single crystals suitable for X-ray crystallography (Scheme 1).

[Ni(L1)]: Yield: 0.242 g (65%); m.p. > 300 °C IR (KBr, cm⁻¹): 2218 (C≡N), 1611 (C=N), 1576 (C=C)_{aliphatic}, 1517 (C=C)_{aromatic}, 1195 (C–O), 754 (Ni–N), 418 (Ni–O). ¹H NMR (250 MHz, d₆-DMSO, ppm): δ = 8.57 (s, 2H, –CH=N), 7.45–6.24 (m, 8H, ArH). UV–Vis spectra [$\lambda_{\max}$, nm (log ϵ , L mol⁻¹ cm⁻¹); DMSO solution]: 265 (4.46), 305 (4.30), 380 (4.25), 440 (4.08), 530 (4.09), 595 (4.08). Anal. Calcd. for C₁₈H₁₀N₄NiO₂: C, 57.9; H, 2.7; N, 15.0%. Found: C, 57.7; H, 2.6; N, 14.9%.

[Ni(L2)]: Yield 0.259 g (60%); m.p. > 300 °C. IR (KBr, cm⁻¹): 2225 (C≡N), 1612 (C=N), 1580 (C=C)_{aliphatic}, 1538 (C=C)_{aromatic}, 1253 (C–O), 735 (Ni–N), 414 (Ni–O). ¹H NMR (250 MHz, d₆-DMSO, ppm): δ = 8.49 (s, 2H,

–CH=N), 7.20–6.45 (m, 6H, ArH), 3.74 (s, 6H, –OCH₃). UV–Vis spectra [$\lambda_{\max}$, nm (log ϵ , L mol⁻¹ cm⁻¹); DMSO solution]: 270 (4.29), 355 (4.16), 375 (4.13), 395 (4.14), 520 (3.97), 600 (4.13). Anal. Calcd. for C₂₀H₁₈N₄NiO₆: C, 51.2; H, 3.8; N, 11.9%. Found: C, 51.0; H, 3.7; N, 11.7%.

Preparation of [Zn(L3)(DMSO)]

This complex was synthesized similar to [Ni(L1)] and [Ni(L2)] complexes. To a solution of 2-hydroxy-1-naphthaldehyde (0.344 g, 2.0 mmol) in DMSO (10 ml), 2,3-diamino-cis-2-butenedinitrile (0.108 g, 1.0 mmol) was added and refluxed for 30 min. A deep brown solution was obtained. After adding triethylamine (0.200 g, 2.0 mmol) and Zn(CH₃CO₂)₂·2H₂O (0.219 g, 1.0 mmol) to the solution, the color of the solution changed to black. Dark red single crystals suitable for crystallography appeared after 2 weeks standing (Scheme 2). Yield 0.407 g (73%); m.p. > 300 °C. IR (KBr, cm⁻¹): 3007 (C–H), 2209 (C≡N), 1601 (C=N), 1571 (C=C)_{aliphatic}, 1536 (C=C)_{aromatic}, 1267 (C–O), 1183 (S=O), 747 (Zn–N), 412 (Zn–O). ¹H NMR (250 MHz, d₆-DMSO, ppm): δ = 9.25 (s, 2H, –CH=N), 8.06–6.94 (m, 12H, ArH), 3.30 (s, 6H, CH₃). UV–Vis spectra [$\lambda_{\max}$, nm (log ϵ , L mol⁻¹ cm⁻¹); DMSO solution]: 265 (4.65), 355 (4.31), 400 (4.40), 425 (4.38), 465 (4.23), 600 (4.71). Anal. Calcd. for C₂₈H₂₀N₄O₃SZn: C, 60.2; H, 3.6; N, 10.0%. Found: C, 60.0; H, 3.3; N, 9.8%.

Scheme 1 A schematic representation of synthesis of [Ni(L1)] and [Ni(L2)] complexes

Scheme 2 A schematic representation of synthesis of [Zn(L3)(DMSO)] complex

X-ray structure determination

Suitable crystals for single-crystal X-ray structure analysis were selected in mineral oil and mounted on glass fibers. Diffraction data for [Ni(L1)], [Ni(L2)] and [Zn(L3)(DMSO)] were collected on an IPDS-1 diffractometer (Stoe&Cie GmbH, Darmstadt, Germany) using graphite-monochromatized Mo-K α radiation, ($\lambda = 0.71073$ Å). The structures were solved by direct methods using SHELXS and refined using SHELXL [17]. All non-hydrogen atoms with the exception of the disordered S atom of the DMSO ligand in [Zn(L3)(DMSO)] were refined anisotropically. Hydrogen atoms were included in idealized positions. The molecular graphics were drawn with DIAMOND [18]. Crystallographic data and details of the data collection and structure refinement are listed in Table 1, selected bond lengths and angles are presented in Table 2, and Scheme 3 shows packing views of the three complexes.

Microorganism strains and culture

In this research, we used one yeast (*Candida albicans* PTCC 5027), five standard bacteria (*Pseudomonas aeruginosa*

ATCC 27853, *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1330, *Bacillus cereus* PTCC 1015 and *Micrococcus luteus* PTCC 1110) and three bacteria isolated from clinical samples (*Klebsiella pneumoniae*, *Enterococcus faecalis* and *Listeria monocytogenes*). Brain Heart Infusion medium (BHI-Merck) was used for *L. monocytogenes* and *E. faecalis*, whereas the other bacteria were cultured on Mueller–Hinton medium (Merck). Also, YGC medium (Merck) was used as the test medium for the yeast strain.

Determination of antimicrobial activity

The antimicrobial activities of [Ni(L1)], [Ni(L2)] and [Zn(L3)(DMSO)] complexes, NiCl $_2$ ·6H $_2$ O and Zn(CH $_3$ CO $_2$) $_2$ ·2H $_2$ O against some Gram positive bacteria strains (*L. monocytogenes*, *E. faecalis*, *S. aureus* PTCC 1112, *M. luteus* PTCC 1110 and *B. cereus* PTCC 1015), some Gram negative bacterial strains (*E. coli* PTCC 1330, *K. pneumoniae*, *P. aeruginosa* ATCC 27853) and a fungal species (*C. albicans* PTCC 5027) were investigated using agar well diffusion plate methods [19]. The agar media were inoculated with 100 μ l of the inoculums which were prepared using an overnight culture of each

Table 1 Crystallographic data collection parameters for [Ni(L1)], [Ni(L2)] and [Zn(L3)(DMSO)]

Complex	[Ni(L1)]	[Ni(L2)]	[Zn(L3)(DMSO)]
Formula	C $_{18}$ H $_{10}$ N $_4$ NiO $_2$	C $_{20}$ H $_{18}$ N $_4$ NiO $_6$	C $_{28}$ H $_{20}$ N $_4$ O $_3$ SZn
Formula weight, g mol $^{-1}$	373.01	469.09	557.91
Crystal size, mm 3	0.30 $\times$ 0.10 $\times$ 0.05	0.10 $\times$ 0.10 $\times$ 0.08	0.20 $\times$ 0.20 $\times$ 0.10
Crystal color	Blue	Blue	Red
Temperature, K	213	213	213
Crystal system	Orthorhombic	Monoclinic	Triclinic
Space group	<i>Pbcn</i>	<i>I2/a</i>	<i>P</i> $\bar{1}$
Unit cell dimensions (Å, °)			
<i>a</i>	17.133 (2)	13.291 (1)	10.4479 (5)
<i>b</i>	6.9752 (4)	13.0637 (6)	11.1601 (5)
<i>c</i>	26.088 (2)	22.605 (2)	11.8326 (6)
α	90	90.00	102.406 (6)
β	90	102.322 (7)	100.556 (6)
γ	90	90.00	111.140 (5)
Volume, Å 3	3117.6 (4)	3834.5 (5)	1204.22 (11)
<i>Z</i>	8	8	2
Calculated density, g cm $^{-3}$	1.589	1.625	1.539
Absorption coefficient, mm $^{-1}$	1.264	1.061	1.146
θ range for data collection, °	2.0–25.0	2.2–25.0	2.1–25.9
Reflections collected	18,304	12,828	11,951
Independent reflections (<i>R</i> $_{int}$)	2681 (0.146)	3335 (0.165)	4372 (0.0273)
Observed reflections [<i>I</i> $\geq$ 2 σ (<i>I</i>)]	1003	1617	3683
Parameters	226	280	344
<i>R</i> 1 (observed reflections)	0.0445	0.0786	0.031
<i>wR</i> 2 (all data)	0.1028	0.1825	0.078
Largest diff. peak/hole, eÅ $^{-3}$	0.47/– 0.84	0.74/– 0.72	0.49/– 0.30

Table 2 Selected bond lengths (Å) and angles (°) of [Ni(L1)], [Ni(L2)] and [Zn(L3)(DMSO)]

[Ni(L1)]					
Ni1–N1	1.854 (6)	N1–C5	1.320 (9)	Ni1–O2	1.834 (5)
Ni1–N2	1.862 (6)	N2–C2	1.406 (8)	N1–C1	1.387 (7)
Ni1–O1	1.836 (5)	C5–C6	1.400 (11)	C1–C2	1.349 (7)
N1–Ni1–N2	86.2 (3)	O2–Ni1–N2	94.19 (16)	O1–Ni1–O2	85.0 (2)
N1–Ni1–O1	94.71 (15)	Ni1–N1–C1	112.0 (5)	O1–Ni1–N2	177.9 (2)
N1–Ni1–O2	178.4 (2)	Ni1–N1–C5	127.5 (5)	N1–C5–C6	124.2 (7)
[Ni(L2)]					
Ni1–N1	1.838 (6)	N1–C5	1.327 (9)	Ni1–O2	1.849 (5)
Ni1–N2	1.860 (6)	C5–C6	1.382 (9)	N1–C1	1.366 (9)
Ni1–O1	1.846 (4)	C1–C2	1.341 (10)		
N1–Ni1–N2	86.0 (3)	O2–Ni1–N2	94.1 (2)	O1–Ni1–O2	85.9 (2)
N1–Ni1–O1	94.0 (2)	Ni1–N1–C1	112.5 (5)	O1–Ni1–N2	178.8 (3)
N1–Ni1–O2	179.9 (3)	Ni1–N1–C5	127.5 (5)	N1–C5–C6	124.1 (8)
[Zn(L3)(DMSO)]					
Zn1–N1	2.0813 (14)	Zn1–O3	2.0430 (16)	N3–C3	1.135 (3)
Zn1–N2	2.0992 (17)	N1–C1	1.378 (3)	C1–C2	1.371 (3)
Zn1–O1	1.9530 (16)	N1–C5	1.319 (2)	C1–C3	1.450 (3)
Zn1–O2	1.9742 (13)	N2–C2	1.386 (2)	C5–C6	1.404 (3)
N1–Zn1–O1	87.43 (6)	O1–Zn1–O3	100.55 (8)	C1–N1–C5	121.68 (16)
N1–Zn1–O2	146.31 (7)	O1–Zn1–N2	159.77 (7)	Zn1–N1–C1	111.94 (12)
N1–Zn1–O3	108.81 (7)	O2–Zn1–O3	102.96 (6)	Zn1–N1–C5	126.06 (13)
N1–Zn1–N2	79.12 (6)	O2–Zn1–N2	85.71 (6)	N1–C5–C6	126.17 (17)
O1–Zn1–O2	97.67 (6)	O3–Zn1–N2	98.08 (8)		

Scheme 3 The packing view of [Ni(L1)] (a), [Ni(L2)] (b) and [Zn(L3)(DMSO)] (c)

microorganism (18–24 h) adjusted to a turbidity equivalent to a 0.5-McFarland standard. Wells were cut and 50 μ l of the compounds (10 mg/ml; DMSO was used as solvent) were added. Each compound was tested in triplicate along with standard ciprofloxacin for bacteria and fluconazole for yeast. The plates were incubated at 37 °C for 24 h. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The diameter of the zone of inhibition was measured by measuring scale in millimeters (mm). DMSO as solvent

was used as a negative control. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by microdilution assay (NCCLS, 2008). The cultures were prepared in 24 and 72 h broth cultures of microorganisms, respectively. The MIC was defined as the lowest concentration of compound to inhibit the growth of microorganisms, and the MBC was defined as the lowest concentration of compound to kill the microorganisms. Serial dilutions ranging from 10 mg/ml to 39 μ g/ml were prepared in medium.

Results and discussion

Syntheses and spectroscopic characterization

In general, metal complexes [Ni(L1)], [Ni(L2)] and [Zn(L3)(DMSO)] were obtained from the condensation of DAMN with various aldehydes in the presence of metal atoms. The IR spectra of the complexes exhibit absorption bands at 1612, 1611 and 1601 cm^{-1} respectively, which are assigned to the (C=N) stretching vibration [20]. The absence of an OH stretching vibration suggests that the O phenolic atoms of the ligands are deprotonated and participate in coordination to the nickel and zinc atoms [21]. The symmetric diimine nature of the complexes is proven by the single band observed in the range of 2209–2225 cm^{-1} assigned to the (C≡N) stretching vibration, contrary to two bands observed in monoimine compounds [22]. The absorption bands in the range of 735–754 cm^{-1} and 412–418 cm^{-1} in the metal complexes are assigned to stretching modes of the M–N and M–O bonds, respectively [23]. In the ^1H NMR spectra of the complexes, the signal at 9.25, 8.57 and 8.49 ppm respectively is attributed to azomethine protons [15]. The electronic spectra of the complexes were recorded in DMSO. The bands at 265 and 270 nm are attributed to the electronic transitions $\pi \rightarrow \pi^*$ of the aromatic rings. The bands between 305 and 400 nm are due to $n \rightarrow \pi^*$ transitions. The bands above 400 nm are attributed to the intense charge transfer and intraligand transitions, indicating efficient conjugation in the metal complexes [24–26].

Fig. 1 ORTEP view of [Ni(L1)]; thermal ellipsoids are drawn at the 50% probability

Structure description of [Ni(L1)] and [Ni(L2)]

The molecular structure of [Ni(L1)] in solid state is shown in Fig. 1. This complex crystallizes in the orthorhombic space group *Pbcn*. The Schiff base ligand coordinates to one nickel atom in a tetradentate manner via phenolate O and imine N atoms. The geometry around the nickel atom is square planar, and the angle sum in the nickel plane is 360.1°. The bond distances between Ni(1) and O are 1.836(5) and 1.834(5) Å, and the Ni(1)–N bond lengths are 1.854(6) and 1.862(6) Å [27].

The molecular structure of [Ni(L2)] in solid state is shown in Fig. 2. [Ni(L2)] crystallizes in the monoclinic space group *I2/a*. The nickel atom is also four-coordinated with a square planar geometry. The coordination environment of the nickel atom is very similar to that of the [Ni(L1)].

Structure description of [Zn(L3)(DMSO)]

The molecular structure of [Zn(L3)(DMSO)] in the solid state is shown in Fig. 3. This complex crystallizes in the triclinic space group *P* $\bar{1}$. The zinc atom is surrounded by two nitrogen atoms and two oxygen atoms from the Schiff base ligand and one oxygen atom from a DMSO ligand. The zinc atom is five-coordinated with a square pyramidal coordination geometry. The tetradentate ligand occupies the equatorial plane, and one O-coordinating DMSO ligand (sulfur atom disordered on two positions with 87.8(2) and 12.2(2)%) occupies the apical position. The coordinating N and O atoms form a plane with a maximum deviation of 0.1254(9) Å. Zn(1) is positioned 0.4381(9) Å below this plane (Fig. 3), the angle sum in the zinc plane amounts to

Fig. 2 ORTEP view of [Ni(L2)] (50% ellipsoids)

Fig. 3 ORTEP view of [Zn(L3)(DMSO)] (50% ellipsoids)

349.9°. The average bond length of Zn–N is 2.0902 Å. The Zn(1)–O(3) bond distance [2.0430(16) Å] to the DMSO ligand is slightly longer than those of Zn(1)–O(1) and Zn(1)–O(2) to the tetradentate ligand (av, 1.9636 Å) [28–30].

Antimicrobial activities

[Ni(L1)], [Ni(L2)] and [Zn(L3)(DMSO)] as well as $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$ were tested for their

in vitro antimicrobial activities (Table 3). Results showed that all three complexes have potential as antimicrobial agents. The complex [Ni(L1)] has activity against three Gram positive bacteria. The rest of the compounds show no selectivity between Gram positive and Gram negative bacterial strains. $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and complex [Ni(L2)] have an antifungal effect against *Candida albicans* PTCC 5027. As shown in Table 3, while *E. faecalis* and *L. monocytogenes* isolated from clinical samples are resistant to the antibiotics

Table 3 In vitro antimicrobial activity of the compounds, 10 mg/ml (IZ)

Microorganism	Inhibition zone (mm)						
	[Ni(L1)]	[Ni(L2)]	[Zn(L3) (DMSO)]	NiCl ₂ ·6H ₂ O	Zn(CH ₃ CO ₂) ₂ ·2H ₂ O	Ciprofloxacin	Fluconazole
<i>P. aeruginosa</i>	0	10	11	9	9	22	0
<i>K. pneumonia</i> ^a	0	0	0	11	11	0	0
<i>E. faecalis</i> ^a	8	11	0	9	8	0	0
<i>S. aureus</i>	9	9	0	8	9	25	0
<i>E. coli</i>	0	16	0	9	10	27	0
<i>B. cereus</i>	0	10	0	10	18	24	0
<i>M. luteus</i>	10	8	7	10	16	31	0
<i>C. albicans</i>	0	9	0	11	0	0	35
<i>L. monocytogenes</i> ^a	0	9	8	8	10	0	0

^aBacteria isolated from clinical samples

ciprofloxacin and fluconazole, *E. faecalis* is sensitive to [Ni(L1)], [Ni(L2)], NiCl₂·6H₂O and Zn(CH₃CO₂)₂·2H₂O and *L. monocytogenes* is sensitive to [Ni(L2)], [Zn(L3) (DMSO)], NiCl₂·6H₂O and Zn(CH₃CO₂)₂·2H₂O. The results of minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) experiments are shown in Table 4. As can be seen, the MIC and MBC values for [Ni(L1)] and [Ni(L2)] are considerably lower than that for NiCl₂·6H₂O and also, for [Zn(L3)(DMSO)] is lower than that for Zn(CH₃CO₂)₂·2H₂O. It means that low concentrations of the complexes not only inhibit the growth of microorganisms, but also kill them. So, they have both bacteriostatic and bactericidal effects.

Conclusion

In conclusion, three metal Schiff base complexes have been synthesized and structurally characterized. The results indicated that the ligands were N₂O₂-tetradentate coordinated

to the metal atoms through both the azomethine N atoms and phenolic O atoms. The nickel(II) complexes had four-coordinate square planar geometries, while the zinc(II) complex was five-coordinated with square pyramidal coordination geometry. In the zinc(II) complex, the tetradentate ligand occupied the equatorial plane and one O-coordinating DMSO ligand occupied the apical position. The antimicrobial activities of the three complexes and NiCl₂·6H₂O and Zn(CH₃CO₂)₂·2H₂O were also studied against different microorganisms. The results showed that all three complexes have some potential as antimicrobial agents. Also, according to the results of MIC and MBC, these complexes have both bacteriostatic and bactericidal effects. Further studies on the antimicrobial activities of diaminomaleonitrile-based complexes are planned for the future.

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Table 4 In vitro antimicrobial activity of the compounds, (MIC and MBC, mg/ml)

Microorganisms	[Ni(L1)]		[Ni(L2)]		[Zn(L3) (DMSO)]		NiCl ₂ ·6H ₂ O		Zn(CH ₃ CO ₂) ₂ · 2H ₂ O	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>P. aeruginosa</i>	–	–	2.5	5	1.25	–	2.5	5	0.156	2.5
<i>K. pneumonia</i>	–	–	–	–	–	–	1.25	2.5	2.5	10
<i>E. faecalis</i>	2.5	5	1.25	5	–	–	5	10	0.625	5
<i>S. aureus</i>	0.625	2.5	2.5	5	–	–	5	10	2.5	5
<i>E. coli</i>	–	–	0.625	2.5	–	–	2.5	5	0.625	2.5
<i>B. cereus</i>	–	5	2.5	5	–	–	5	10	0.039	1.25
<i>M. luteus</i>	0.16	2.5	1.25	2.5	0.13	1.25	1.25	5	0.156	2.5
<i>C. albicans</i>	–	–	1.25	2.5	–	–	2.5	5	–	–
<i>L. monocytogenes</i>	–	–	2.5	5	0.25	2.5	5	10	0.625	2.5

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